

Identification and Activity-Dependent Labeling of Peripheral Sensory Structures on a Spionid Polychaete

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Abstract. In marine sedimentary habitats, chemoreception is thought to coordinate feeding in many deposit-feeding invertebrates such as polychaetes, snails, and clams. Relatively little is known, however, about the chemosensory structures and mechanism of signal transduction in deposit feeders. Using electron microscopy, confocal laser scanning microscopy (CLSM), and immunohistochemistry, we investigated the structure and function of putative chemosensory cells on the feeding appendages of a deposit-feeding polychaete species, *Dipolydora quadrilobata*. Tufts of putative sensory cilia were distributed over the prostomium and feeding palps and typically occurred next to pores. Examination of these regions with transmission electron microscopy revealed multiciliated cells with adjacent glandular cells beneath the pores. The sensory cells of prostomium and palps were similar, displaying an abundance of apical mitochondria and relatively short ciliary rootlets. Staining with antiserum against acetylated α -tubulin was examined by CLSM, and revealed axonal processes from putative sensory tufts on the palp surface to palp nerves, as well as many free nerve endings. Activity-dependent cell labeling experiments were used to test the sensitivity of putative sensory cells on the palps to an amino acid mixture that elicited feeding in previous behavioral experiments. In static exposures, the number of lateral and abfrontal cells labeled in response to the amino acid mixture was significantly greater than in the controls. Ultrastructural, positional, and now physiological evidence strongly suggests that spionid feeding palps are equipped with sensory cells, at least some of which function as chemoreceptors.

Introduction

In marine sedimentary habitats, deposit-feeding invertebrates such as polychaetes, bivalves, gastropods, crustaceans, holothurians, and hemichordates are abundant members of the macrofauna. These organisms ingest large volumes of sediment with typically low food value, often processing one or more body weights of sediment each day (reviewed in Lopez and Levinton, 1987). They also disturb the sediment as they burrow or build tubes. As bioturbators, deposit feeders have a profound influence on the biological, chemical, geological, and even physical properties of their habitat. Deposit feeders affect sediment transport and distribution (e.g., Nittrouer and Sternberg, 1981; Suchanek, 1983; Shull, 2001), sediment geochemistry (Marinelli, 1992; Aller, 1994), sediment microbial communities (Findlay *et al.*, 1990; Grossman and Reichardt, 1991; Plante and Mayer, 1994), nutrient cycling (Widbom and Frithsen, 1995; Christensen *et al.*, 2000), and fate of pollutants (Mayer *et al.*, 1996; Weston *et al.*, 2000). Sediment disturbance by deposit feeders mediates competitive interactions (e.g., Rhoads and Young, 1970; Woodin, 1976; Wilson, 1981; Brenchley, 1981), and influences animal distribution patterns and dispersal (Wilson, 1981; Gunther, 1992; Brey, 1991), as well as recruitment (Williams, 1980; Posey, 1986; Luckenbach, 1987; Hines *et al.*, 1989; Olafsson, 1989; Flach, 1992). In addition, deposit feeders can influence recruitment directly by ingesting larvae and juveniles (Wilson, 1980; Tamaki, 1985; Miliekovsky, 1974; Elmgren *et al.*, 1986; Albertsson and Leonardsson, 2001).

Rates of sediment mobilization by deposit feeders depend on food supply. In the last two decades, behavioral, physiological, and mathematical modeling approaches have been applied to the question of what makes some sediments better food than others (e.g., review by Jumars, 1993). Largely absent from this body of research, however, are studies that investigate the cues that initiate ingestion and modulate feeding rates in deposit feeders. Jumars (1993)

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suggested several stimuli that could operate to regulate ingestion rate: smell, taste, distension of the gut, and internal detection of the absorbed products in body fluids. The physiological and molecular mechanisms for detecting these stimuli remain poorly understood for most deposit feeders.

Chemoreception is implicated in the coordination of feeding by a variety of deposit feeders. For example, fresh fecal material depresses feeding rate in the snail *Hydrobia trincata* (Forbes and Lopez, 1986) and the spionid polychaete *Pseudopolydora kempii japonica* (Miller and Jumars, 1986). Phagostimulants are implicated as well. When given a choice, the common deposit-feeding polychaete *Streblospio benedicti* fed preferentially on organically enriched sediments rather than on unaltered sediments (Kihlslinger and Woodin, 2000). Feeding rate in a deposit-feeding hemichordate was strongly correlated with sediment chlorophyll *a* concentrations (Karrh and Miller, 1994). Robertson *et al.* (1980) found that fiddler crabs, *Uca pugilator*, fed selectively in diatom-enriched patches of sediment and could resolve patches at the millimeter scale by probing with sensory setae on their legs. Fiddler crabs have also been the focus of several studies that explicitly tested the effects of chemical compounds on deposit feeders. A variety of amino acids, peptides, and sugars appear to stimulate feeding (Robertson *et al.*, 1981; Rittschof and Buswell, 1989; Weissburg and Zimmer-Faust, 1991; Weissburg, 1993). Similarly, Ferner and Jumars (1999) found that dissolved cues (amino acids and complex mixtures) influenced the feeding behavior of several spionid polychaetes. We recently extended this work to show that particle-bound amino acids and sugars influence feeding in the spionid polychaete *Dipolydora quadrilobata* (Riordan and Lindsay, 2002).

Although chemical mediation of deposit feeding seems likely, relatively little is known about the chemosensory structures and mechanism of signal transduction in deposit feeders. One exception is fiddler crabs. Weissburg and colleagues have identified gender-specific differences in fiddler crab feeding behavior in response to chemical cues, and these are linked to differences in the number and sensitivity of chemoreceptor neurons (Weissburg, 1993, 1999; Weissburg and Derby, 1995; Weissburg *et al.*, 1996). Recent work (Weissburg, 2001) suggests the adenylate-cyclase-cAMP second messenger cascade mediates inhibition of chemosensory neurons, and that gender-specific differences in this pathway contribute to the physiological and behavioral differences in fiddler crab chemosensitivity.

Among the polychaetes, nuchal organs are presumed to be involved in chemoreception based on histological, ultrastructural, and positional criteria (Storch and Schlötzer-Schrehardt, 1988; Purschke, 1997). Several authors have speculated that nuchal organs may be involved in food selection (Rullier, 1951; Rhode, 1990; Nozais *et al.*, 1997) or reproduction (Schlötzer-Schrehardt, 1987), although

they may also be involved in osmoregulation (Fewou and Dhainaut-Courtois, 1995). Nuchal organs typically are paired epidermal structures found on the dorsal side of the prostomium or peristomium (*i.e.*, the anterior presegmental region). Some spionid polychaetes (not *Dipolydora quadrilobata*) also have metameric nuchal organs on their bodies (Jelsing, 2002). Cephalic nuchal organs are typically composed of ciliated supporting cells, bipolar primary sensory cells with cilia, unmodified epidermal cells, and retractor muscle cells in those species in which nuchal organs can be retracted (reviewed by Purschke, 1997).

Other presumed chemosensory structures have been described from polychaetes, including epidermal papillae of the deposit-feeding lugworm *Arenicola marina* (Jouin *et al.*, 1985), compound sensory organs on the prostomial cirri and palps of *Nereis diversicolor* (Dorsett and Hyde, 1969), and the parapodial cirri of nereidid polychaetes (Boilly-Marer, 1972). In *Platynereis dunerlii*, the receptors of the parapodial cirri function in perception of sexual pheromones (Boilly-Marer, 1968, 1974). Peripheral sensory structures have also been observed on the feeding palps, prostomia, and peristomia of several spionid polychaete species (Dauer, 1984, 1987, 1991, 1997; Worsae, 2001). With the exception of the nereidid pheromone receptors, studies demonstrating functions of these putative polychaete chemoreceptors are largely absent. The goals of this study were to (1) describe the distribution and ultrastructure of peripheral sensory cells of the spionid polychaete *Dipolydora quadrilobata*, and (2) assign a functional role to these structures by using an immunohistochemical approach that labeled cells responding to chemical cues that elicited behavioral responses in *D. quadrilobata* (Riordan and Lindsay, 2002).

Materials and Methods

Collection and maintenance of animals

Individuals of *Dipolydora quadrilobata* (Jacobi 1883) were sieved (0.5 mm) out of cores collected from the mudflats of Lowe's Cove at the University of Maine's Darling Marine Laboratory (Walpole, ME, USA) on several days in September and October of 2000, and March, April, and May of 2001. Animals and natural sediments were transported to the University of Maine in Orono and maintained in aquaria inside an environmental chamber (14 °C:10 °C, 12 h light:dark cycle). Individual worms that measured 10–20 mm in length and showed no signs of gametogenesis, loss of segments, or other bodily damage were used in the experiments.

Electron microscopy

For scanning electron microscopy (SEM), intact worms were relaxed in chilled 37% magnesium chloride, fixed in

3% glutaraldehyde in 0.1 M phosphate buffer with 10% sucrose, post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, and dehydrated in an ethanol series. Samples were then critical-point-dried with liquid CO₂, mounted on stubs, and coated with gold palladium in a Conductvac 1 (SpecVac, Inc.) sputter coater. Samples were viewed with an AMRay AMR1000A scanning electron microscope operating at 5 kV. Negatives were scanned at 800 dpi with an HP Scanjet 7400C flatbed scanner equipped with an HP Scanjet XPA attachment, and the images were saved as TIFF files.

For transmission electron microscopy (TEM), worms were relaxed in chilled 37% magnesium chloride, fixed in 3% glutaraldehyde in phosphate buffer with 10% sucrose, post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated in an acetone series, and embedded in Spurr's resin. Ultrathin sections were collected onto slot grids, then stained with 0.5% lead citrate and 2% uranyl acetate. Samples were viewed with a Phillips CM10 transmission electron microscope operating at 80 kV. Negatives were scanned at 800 dpi with an HP Scanjet 7400C flatbed scanner equipped with an HP Scanjet XPA attachment, and the images were saved as TIFF files.

Confocal laser scanning microscopy

Worms were relaxed in a 37% MgCl₂ solution for 5 min, then fixed overnight at 4 °C in a solution of 4% formaldehyde in artificial seawater (ASW, 32‰, pH 7.4, Forty Fathoms Crystal Sea Marine Mix, Marine Enterprises International, Baltimore, MD). After three 10-min rinses in ASW, worms were soaked for 5 h in a blocking solution containing 0.5% Triton-X 100 and 0.5% bovine serum albumin (BSA). The primary antibody (1° Ab), monoclonal mouse anti-acetylated α -tubulin (Sigma, St. Louis; clone 6-11B-1), was diluted 1:100 with 0.5% Triton X-100 in ASW and applied overnight (about 18 h). Specimens were then rinsed in ASW and incubated for 5 h with FITC-conjugated goat anti-mouse secondary antibodies (Sigma; Fc specific IgG) diluted 1:100 in 0.5% Triton X-100/ASW. After a final series of ASW rinses, worms were mounted on glass slides in Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL). Preparations were imaged with a Leica TCS SP2 confocal laser scanning microscope. Negative controls to test for the specificity of primary antibodies were prepared by treating specimens as described, but omitting the 1° Ab. Repeated incubations were performed, and at least 10 worms were used to test for reproducibility.

Activity-dependent cell labeling

We adapted the method used by Michel *et al.* (1999) to label activated olfactory neurons in zebrafish and spiny lobsters. This method exploits the ability of cationic guanidinium analogs to enter into stimulated neurons and meta-

bolically active cells (Dwyer *et al.*, 1980; Picco and Menini, 1993). These analogs enter active neurons through nonspecific cation channels activated and opened by the binding of a ligand with its receptor protein. Sequestration of the analogs in these cells allows for the activity-dependent labeling of individual receptor neurons.

For example, the guanidinium analog, 1-amino-4-guanidobutane (= agmatine), has been shown to enter into receptor neurons through such open cation channels (Yoshikami, 1981). When coupled with known stimulatory cues in solution and perfused over olfactory organs, agmatine accumulates in activated odorant receptor neurons (Michel *et al.*, 1999; Steullet *et al.*, 2000). Cells stimulated by a cue accumulate agmatine and can be identified using an anti-agmatine IgG antibody followed by silver intensification labeling (Mare, 1995, 1999a, b). We presented agmatine plus a mixture of amino acids known to elicit behavioral responses to individual specimens of *Dipolydora quadrilobata* in both flow-through and static experiments.

Flow trials. Individual worms were immersed in artificial seawater (ASW, in mM: 423 NaCl, 9 KCl, 13 CaCl₂, 23 MgCl₂, 26 MgSO₄ (Cavanaugh, 1975) pH adjusted to 7.2) inside a small coverslip perfusion chamber (Warner Instruments, Model #RC 21B). Odorant stimuli were added to the ASW perfusion fluid in 5-s pulses every 60 s for 60 min. The ASW and the odorant stimuli solutions were held in 60-ml syringes connected to the perfusion chamber through rubber tubing and a manifold. Fluid flow from the syringes was by gravity feed, and flow rates (0.5 cm s⁻¹) were controlled by stopcock valves; fluid flow was turned on and off by electronically activated pinch valves (Warner Instruments, model VC-6). Stimuli included 20 mM agmatine sulfate (AGB) in ASW (control, *n* = 4 worms) and 20 mM AGB plus a mixture of amino acids (1 mM each of proline, alanine, threonine, valine, taurine, and glycine) in ASW (treatment, *n* = 5 worms). Following the 60-min stimulation period, ASW was perfused over the worms for 5 min to remove residual AGB. Worms were then immersed in fresh ASW and relaxed by placing them in a freezer (-20 °C) for 10 min prior to fixing. Whole worms were placed in fixative (1% paraformaldehyde, 2% glutaraldehyde in 0.2 M phosphate buffer with 10% sucrose (w/v), pH 7.2) from overnight to several days.

No flow (static) trials. Individual worms were immersed in 10 ml ASW inside a small petri dish, and 10 ml of either 40 mM AGB in ASW (control, *n* = 6 worms) or 40 mM AGB plus the mixture of amino acids in ASW (treatment, *n* = 6 worms) was slowly added by pipette. Worms were exposed to the treatments for 60 min, during which time they were relatively quiescent in the dishes, immersed in fresh ASW for 5 min to remove residual AGB, and relaxed by placing them in a freezer (-20 °C) for 10 min prior to fixing. Whole

worms were placed in fixative (1% paraformaldehyde, 2% glutaraldehyde in 0.2 M phosphate buffer with 10% sucrose (w/v), pH 7.2) from overnight to several days.

Tissue processing, immunolabeling, and visualization

Fixed worms were rinsed in a phosphate buffer (PB: 1.76 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ + 7.67 g Na_2HPO_4 in 1 l of deionized water), dehydrated through a graded series of absolute ethanol and acetone, embedded in Epon 812 resin, cured, and sectioned using a microtome and glass knives. An average of 156 sections were processed for each individual. Semi-thick sections (2 μm) were placed in 7-mm wells of a Teflon-coated spot slide (Eric Scientific), deplasticized in a 1:5 v/v solution of mature sodium ethoxide in anhydrous ethanol, and subsequently washed in three changes of anhydrous ethanol. The slides were rinsed in deionized water, air-dried, and then incubated overnight in a 1:100 dilution of a polyclonal rabbit anti-AGB IgG antibody (Signature Immunologics and courtesy of R. Marc, University of Utah School of Medicine). The slides were then rinsed in PB, washed in 1% goat serum in PB plus 0.05% thimerosal (1% GSPBT) for 10 min, and incubated in a 1:50 dilution of a 1-nm gold conjugated anti-rabbit IgG antibody for 60 min. After a final PB wash and air-drying, labeled cells were visualized using silver intensification (Marc, 1999a, b). The silver nitrate solution for silver intensification was prepared by mixing three solutions: 5 ml of solution A (114 mg citric acid + 342 mg sodium citrate in 6 ml of deionized water), 1 ml of solution B (0.5 g hydroquinone in 15 ml of deionized water), and 1 ml of 1% aqueous silver nitrate. Following a dip in 5% acetic acid to stop the intensification reaction, the slides were washed in deionized water for 10 min, air-dried, and mounted in Permount (Fisher) for viewing on a light microscope.

Image digitization and analysis

Images of the sections were captured digitally using a Javelin JE12HMV video camera mounted on an Olympus BX60 light microscope. Video signals were passed to a frame grabber board (Scion LG 3) in a Dell Optiplex GX110 computer. The images were analyzed using the Scion ImagePC software, Beta ver. 4.02 (Scion Corporation, Frederick, MD). Cells labeled with agmatine were identified by quantifying the pixel intensity inside a cell of interest in the digitized images and comparing it to the pixel intensity of an unlabeled region adjacent to the cell (e.g. Michel *et al.*, 1999). The mean and standard deviation of the pixel intensity inside cells of interest was used to calculate a 95% confidence interval; cells were counted as labeled if the lower limit of this interval was higher than the upper limit of the unlabeled region. Two strategies were used to avoid double-counting of labeled cells. First, cells were counted only if the distal processes and apical cilia were

present in the same plane as the labeled cell body; these processes typically were not coplanar in more than one adjacent section. Second, the locations of labeled cells in a single section were compared to labeled cells in adjacent sections to avoid counting the same cell twice.

Labeled cells were grouped by type according to their location within the sections (Fig. 1). Abfrontal cells were located behind a straight line drawn across the sections and tangent to the back of the food groove. Lateral cells were found between the food groove and the abfrontal cilia. Cells immediately adjacent to the frontal ciliated food groove were called laterofrontal cells. And cells located within the frontal ciliated food groove were called frontal cells. Because different numbers of sections were analyzed for each worm, we calculated the number of labeled cells per 100 μm palp length, based on the total number of sections analyzed per individual. The resulting data were compared among treatments using a nonparametric Kruskal-Wallis analysis of variance (SAS version 9, SAS Institute, Inc.). Data from flow experiments and static experiments were analyzed separately.

To estimate the total number of each cell type in any length of palp, serial sections made from the palps (middle portion) of four worms were stained with a toluidene blue stain. Toluidene blue stains acidic cell parts (*i.e.*, nucleus) and allows the different types of cells to be identified and counted. Individual cells were traced through the sections to avoid double-counting. On average, about 121 μm of palp tissue was sectioned for each of these worms, representing about 5% of the average length of the palps of *D. quadrilobata*.

Results

Distribution of peripheral sensory cells

Scanning electron microscopy revealed tufts of cilia (*i.e.*, cirri, *sensu* Worsaae, 2001) distributed on the palps and prostomium of *Dipolydora quadrilobata* (Fig. 2). Tufts of putative sensory cilia were found on all surfaces of the prostomium (Fig. 2B) and were typically adjacent to pores (Fig. 2B). The cilia of the prostomial tufts were nonmotile and relatively short; they numbered about 13–20 cilia per tuft (Fig. 2C). On the palps, we observed motile cilia arrayed in a row immediately adjacent to each side of the food groove (laterofrontal, Fig. 3A, B), and tufts of motile cilia arrayed in a second lateral row and on the abfrontal surface (Fig. 3A, B). Laterofrontal, lateral, and abfrontal cilia occurred with similar frequency along the length of the palp (Fig. 3C). The putative sensory tufts of cilia on the abfrontal surface seemed regularly dispersed; and, interestingly, we observed fewer adjacent pores than on the prostomium. The cilia of the abfrontal surfaces were longer than

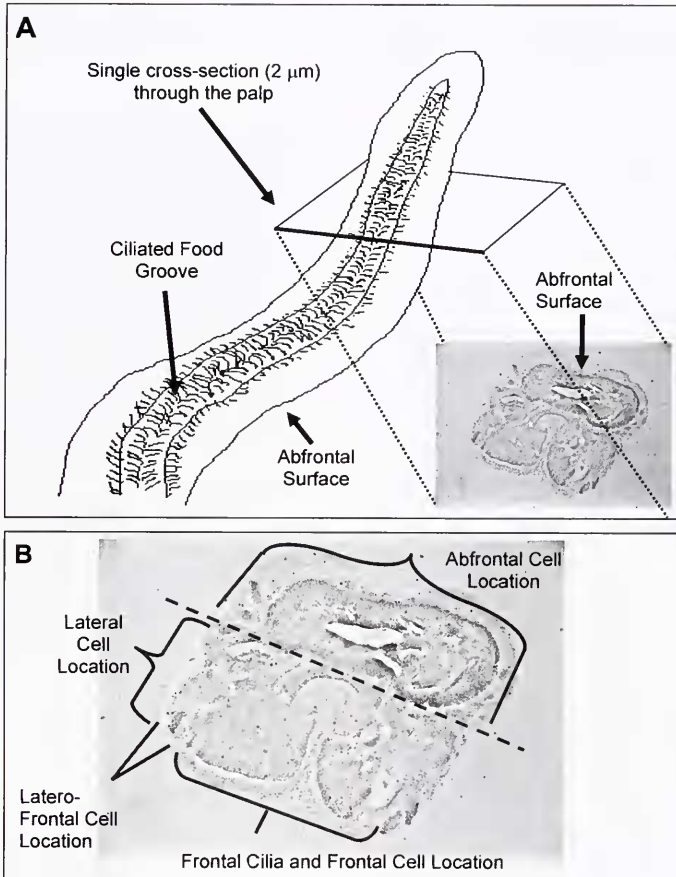


Figure 1. (A) Gross palp morphology and section placement for cell labeling studies in *Dipolydora quadrilobata*. (B) Cell type location within sections. Cells along the food groove and within the frontal cilia were designated frontal cells. Cells immediately adjacent to the frontal cilia were designated laterofrontal cells. Cells behind a line drawn across the back end of the food groove were designated abfrontal cells. Cells in front of that line and lateral to the frontal surface were designated lateral cells.

those of the prostomium, and there were fewer cilia per tuft (7–14) (Fig. 3C, inset).

Sensory cell ultrastructure

Palp and prostomial sensory cells shared similar ultrastructural characteristics. All were multiciliated and contained many apical mitochondria (Figs. 4, 5). Laterofrontal cells on the palp were typically adjacent to glandular cells (Fig. 4) associated with pores on the surface. Gland cells contained either globules of very electron-dense material (Fig. 4C) or less dense material that appeared to be mucus (not shown). Abfrontal cells of the palp were restricted by

the muscle layers below, and cell bodies often projected laterally (Fig. 5). Ciliary rootlets of both palp and prostomial cells, when observed, were short relative to the length of the cell body (Fig. 5A). All cilia were readily distinguishable from the microvillar cuticle (Figs. 4, 5) and displayed a characteristic $9 \times 2 + 2$ microtubular arrangement in cross-section.

Confocal laser scanning microscopy

The antibody for acetylated α -tubulin targets nerve axons and cilia axoneme microtubules. Cilia of the food groove,

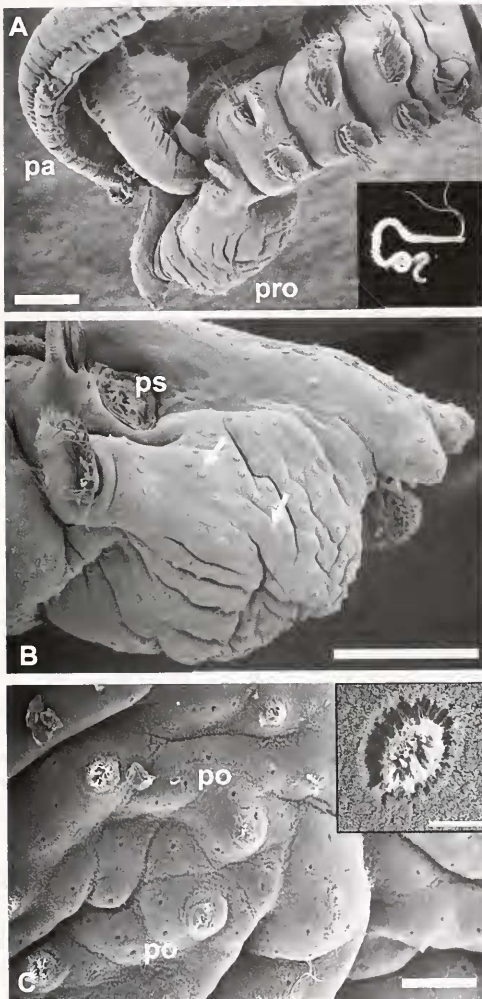


Figure 2. Peripheral sensory cells on the prostomium of *Dipolydora quadrilobata*. (A) Anterior profile, showing palps (pa) and prostomium (pro); scale = 100 μ m. Inset shows entire worm. (B) Ciliated structures are distributed on the entire surface of the prostomium (arrows); palps were removed, leaving a scar (ps); scale = 100 μ m. (C) Pores (po) are found adjacent to the sensory structures on the prostomium; scale = 10 μ m. Inset shows the sensory cilia of the prostomium; inset scale = 2.5 μ m.

lateral and abfrontal surfaces of palp, as well as the main palp nerve and several smaller palp nerves projecting to the central nervous system showed acetylated α -tubulin reactivity (Fig. 6A). Our initial observations clearly show axons that project from the cilia of putative lateral and abfrontal

sensory tufts to the palp nerves (Fig. 6B). There are also many nerves with apparent free endings on the palp surface (*i.e.*, not associated with ciliated cells). A more detailed

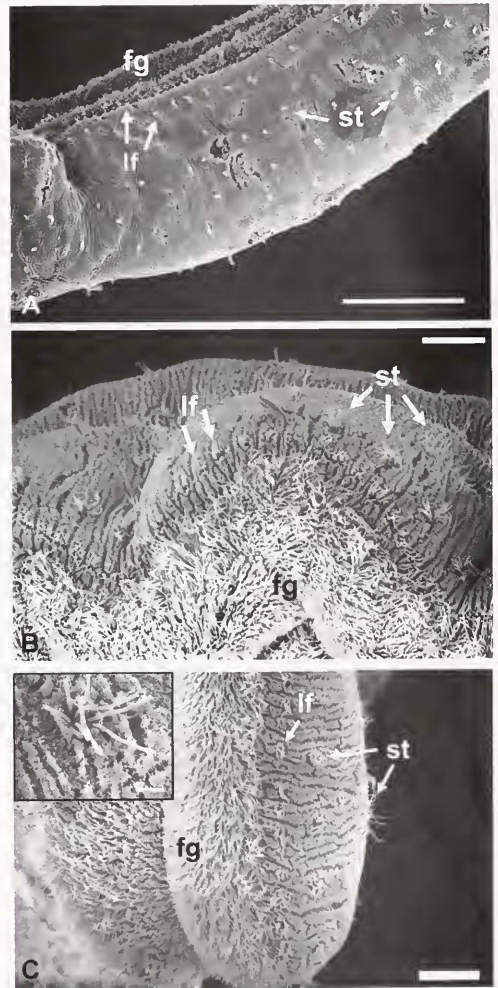


Figure 3. Peripheral sensory cells on the palp of *Dipolydora quadrilobata*. (A) Lateral view of the middle portion of a feeding palp, showing frontal food groove (fg), laterofrontal cilia (lf), and tufts of nonmotile sensory cilia (st) on the lateral and abfrontal surfaces of the palp; scale = 50 μ m. (B) Middle portion of the feeding palp showing laterofrontal (lf) cilia adjacent to the frontal food groove (fg), as well as laterally distributed sensory tufts (st); scale = 10 μ m. (C) Distal portion of feeding palp showing the food groove (fg), laterofrontal (lf) cilia, and sensory tufts (st); scale = 10 μ m. Inset: palp sensory tufts appear to be similar to those on the prostomium; inset scale = 1 μ m.

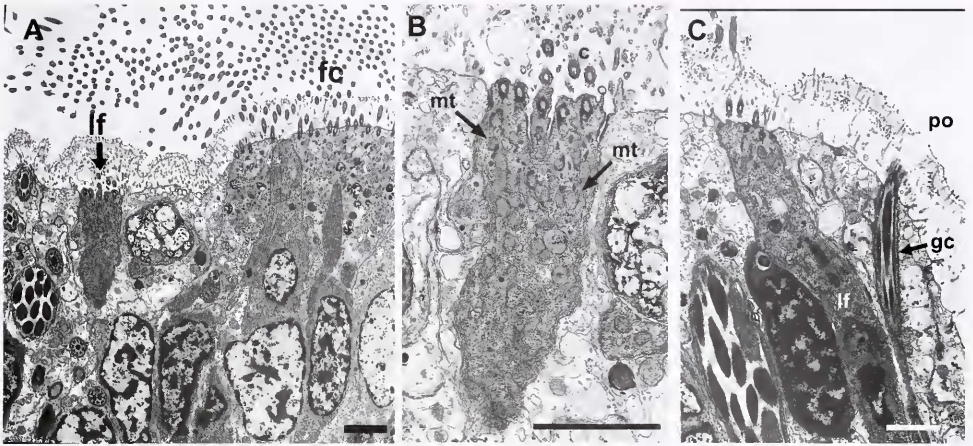


Figure 4. Ultrastructure of *Dipolydora quadrilobata* palp laterofrontal cells. (A) Semi-oblique section through palp food groove showing dense field of frontal cilia (fc) and adjacent laterofrontal cell (lf); scale = 2 μ m. (B) Same laterofrontal cell showing numerous cilia (c) and many apical mitochondria (mt); scale = 2 μ m; the basal portion of the cell projects out of the plane of section. (C) Another semi-oblique section of the palp reveals the laterofrontal cell and adjacent glandular cell (gc) with pore (po); scale = 2 μ m.

examination of the innervation of these peripheral sensory cells, as well as their reactivity to anti-serotonin and anti-FMRamide, is in progress.

Activity-dependent labeling

Four types of putative sensory cells were labeled by exposing the palps of *D. quadrilobata* to agmatine and the amino acid mixture: frontal, laterofrontal, lateral, and abfrontal cells (Fig. 7). These four cell types all have cellular processes extending through the epidermis to the surface of the palp (Fig. 7), many with visible cilia extending from the surface of the palp. Frontal and laterofrontal cells were generally found as groups of several cells close to one another at the periphery of the food groove, and often most of these cells were labeled. In contrast, labeled lateral and abfrontal cells were always found in isolation.

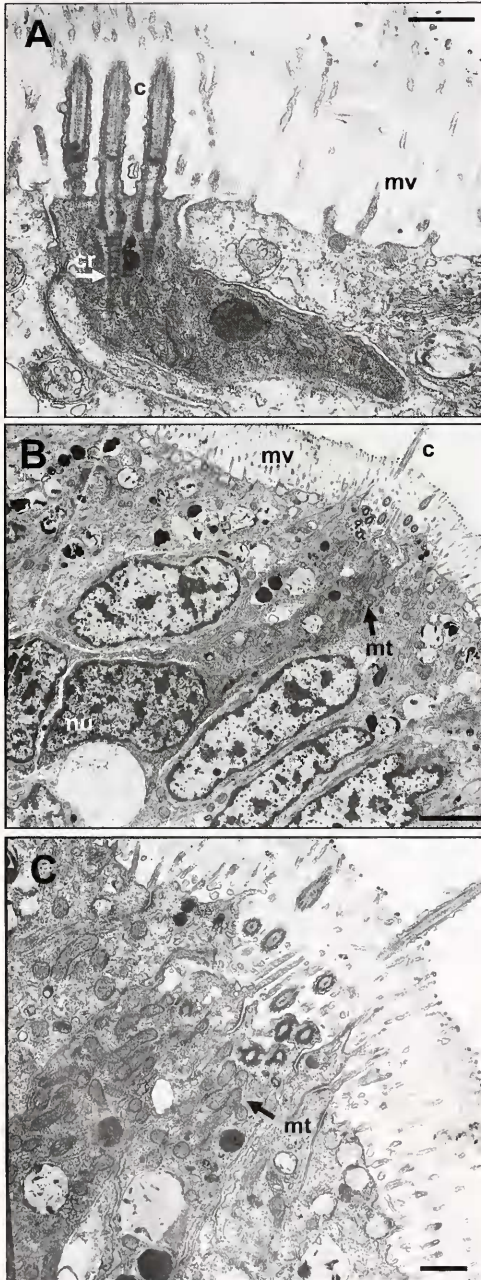
The occurrence of labeled cells in the flow-through perfusion experiment was quite variable (Fig. 8A). Pooling all cell types, there was no significant effect of amino acids (cue) on cell labeling (Kruskal-Wallis $\chi^2 = 0.74$, d.f. = 1, $P = 0.39$, $n = 27$). There was a non-significant trend toward greater labeling in the controls for laterofrontal and frontal cells (e.g., Kruskal-Wallis test of the difference between the number of frontal cells labeled in the presence of amino acids compared to controls: $\chi^2 = 2.7655$, d.f. = 1, $P = 0.09$, $n = 9$). Relatively fewer cells were labeled in the static experiments (Fig. 8B, note axis scale). In contrast to the flow experiment, however, addition of amino acids significantly increased cell labeling with agmatine com-

pared to controls in all cell types pooled (Kruskal-Wallis, $\chi^2 = 9.88$, d.f. = 1, $P = 0.002$, $n = 36$). We observed the greatest amount of labeling in lateral and abfrontal cells (Fig. 8B).

Based on counts from the serial sections stained with toluidine blue, frontal cells and laterofrontal cells occur at about the same frequency, averaging 27 frontal cells and 28 laterofrontal cells per 100 μ m of palp. Lateral and abfrontal cells occur less frequently, at a combined average rate of 11 cells per 100 μ m of palp. Given an average palp length of 2.55 mm for *D. quadrilobata*, and assuming no changes in distribution along the length of the palp, we can then expect approximately 688 frontal cells, 714 laterofrontal cells, and a total of 280 lateral and abfrontal cells to occur on a single palp. Based on the occurrence of labeling that we observed in static trials only, we conservatively estimate that 14% of the total number of lateral and abfrontal cells, 4% of the total number of laterofrontal cells, and 3% of the total number of frontal cells were labeled by agmatine in the presence of amino acids.

Discussion

The diversity of chemosensory organs among marine organisms is great, from crustacean aesthetasc sensilla to molluscan osphradium, rhinophores, and oral tentacles, to polychaete nuchal organs (Laverack, 1968; Emery, 1992). Despite this diversity, the transduction of externally detected signals to the brain follows a similar pathway, beginning with the activation of a chemoreceptor cell that



leads to the central nervous system. The structural common denominator for all chemosensory cells is the presence of ciliary or microvillar extensions into the environment. The peripheral cells of the spionid palps and prostomium clearly meet this requirement (Figs. 2, 3).

Dauer (1984, 1987, 1991, 1997) and Worsae (2001) observed nonmotile cilia on palps and prostomia of several spionid species, including *Dipolydora quadrilobata*, and classified them as sensory on the basis of scanning electron microscopy data and positional criteria. Our initial TEM observations (Figs. 4, 5) support these classifications, and suggest that these peripheral sensory cells have features similar to the caudal chemoreceptors that Jouin *et al.*, (1985) described in *Arenicola marina*—namely an abundance of apical mitochondria and short ciliary rootlets. Confocal laser scanning microscopy further reveals axonal processes to palp nerves from the lateral and abfrontal sensory tufts (Fig. 6), as well as many free nerve endings projecting to the lateral and abfrontal surfaces of the palps.

Assigning function to cells based solely on morphological criteria can be difficult, however. For example, it appears that no single morphological character defines chemoreceptor cilia: on the crustacean aesthetasc, olfactory cilia can have “significant” ciliary rootlets and motile cilia (Grünert and Ache, 1988). We used the activity-dependent labeling experiments to explore the possible function of the palp sensory cells. Although we designed the experiment to assay for cell activity in the presence or absence of chemical cues (amino acids), it is important to note that this method of labeling active sensory cells does not discriminate between types of receptors. It simply identifies cells that have accumulated agmatine, regardless of the stimulus source. Thus, the labeled cells may include a variety of sensory cell types. The initial stimulation of a mechanoreceptor (*i.e.*, stretching or bending of the cell membrane or a protruding cilium) in at least one invertebrate (the crayfish) opens a stretch-activated ion channel that appears to be permeable to divalent cations (Edwards *et al.*, 1981). Chemoreceptor transduction proceeds similarly, with the binding of a ligand to the receptor in the membrane causing the activation and opening of ion channels. In both cases, such cation-permeable channels should be permeable to agmatine, and therefore both mechanoreceptors and chemoreceptors could be labeled using this technique.

Spionid palps are probably equipped with mechanosen-

Figure 5. Ultrastructure of *Dipolydora quadrilobata* palp abfrontal cell and prostomial sensory cells. (A) Constrained by a muscle layer below, this abfrontal palp sensory cell projects laterally. Cilia (c) are readily distinguished from the microvilli (mv) of the cuticle; ciliary rootlets (cr) appear relatively short; scale = 0.5 μm . (B) Prostomial sensory cells have a similar structure, with cilia (c) projecting through the microvillar (mv) cuticle, numerous mitochondria (mt), and a bilobed nucleus (nu); scale = 2 μm . (C) Higher magnification of the same cell, showing apical mitochondria (mt); scale = 0.7 μm .

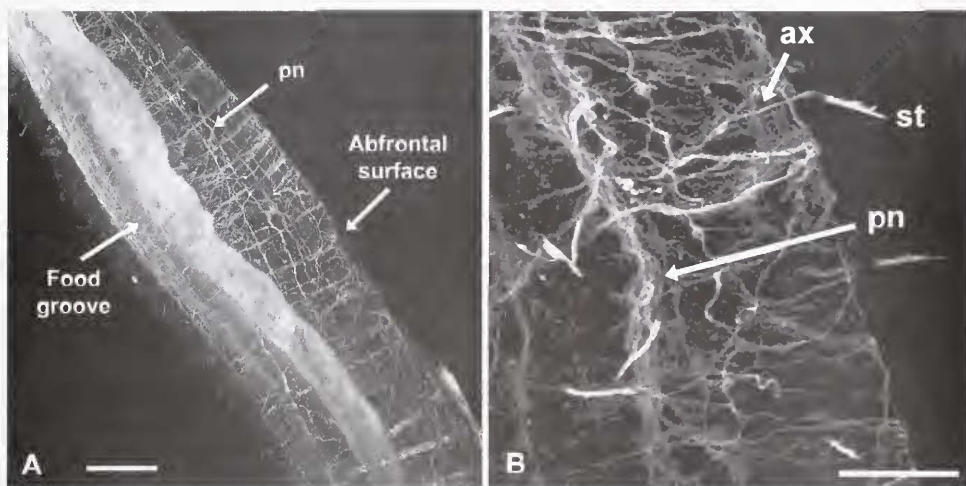


Figure 6. Fluorescence of anti-acetylated α -tubulin in *Dipolydora quadrilobata* palps examined with confocal laser scanning microscopy reveals (A) palp nerves (pn), and cilia of the frontal food groove (scale bar $40\ \mu\text{m}$) as well as (B) cilia of the abfrontal putative sensory tufts (st), their axons (ax), and their proximity to the palp nerves (pn); scale = $25\ \mu\text{m}$).

sory cells. Many spionids (including *D. quadrilobata*; T. Riordan, pers. obs.) switch from deposit feeding to suspension feeding in the presence of higher flow rates (Taghon *et al.*, 1980; Dauer *et al.*, 1981). This switch is probably mediated by mechanosensory detection of flow rates. In addition, when suspension feeding, spionids collect suspended particles that directly intercept the palps. Cilia lining the edge of the frontal food groove (*i.e.*, laterofrontal cilia) and inside the food groove (*i.e.*, frontal cilia) move such particles toward the mouth (Dauer, 1984, 1985, 1987), and may be mechanosensitive. For example, the laterofrontal cilia of one spionid, *Paraprionospio pinnata*, beat only when contacted by a suspended particle (Dauer, 1985), suggesting a mechanosensory influence on activity.

The experiments we conducted did not explicitly isolate mechanical stimulation as a factor affecting cell labeling; thus we cannot make any conclusions about a possible mechanosensory function for the palp sensory cells. However, the trend toward increased labeling of the laterofrontal and frontal cells in the flow-through controls (Fig. 8A) is intriguing, and the possibility that these cells function as mechanoreceptors should be further examined using electrophysiological approaches. It is also possible that the increased labeling in controls was due to the presence of agmatine, which itself is a potent chemical cue in zebrafish (Michel *et al.*, 1999). In the static trials, significantly more cells were labeled in the presence of amino acids and agmatine than in agmatine alone (Fig. 8B); increased labeling was especially pronounced in the lateral and abfrontal

cells. There was virtually no labeling in the agmatine controls. These results suggest that the lateral and abfrontal cells are chemosensory, although it is possible that the cells were stimulated by a response triggered elsewhere on the body. We believe that body movement was an unlikely stimulus, however, because the worms were quiescent during the static trials.

If we accept that the lateral and abfrontal ciliated tufts are chemosensory, then the question arises whether the cells function as distance or contact chemoreceptors. When spionid polychaetes are deposit feeding, they typically probe the sediment surface with the distal portion of the palps (Dauer *et al.*, 1981; Lindsay, pers. obs.), using the abfrontal surface to contact the sediment surface. Previously, we showed that *D. quadrilobata* increased active feeding, including sediment probing, when presented with particle-bound amino acids and sugars (Riordan and Lindsay, 2002). Because a waterborne cue was minimal or nonexistent, we concluded that the palps mediated the response. In addition, Ferner and Jumars (1999) reported repeated "pore-water-sniffing" behavior by *Boccardia proboscidea* in which the palps were raised into the water column, sank passively onto the sediment, and then quickly returned to an upright position. Again, it was the abfrontal palp surface that contacted the sediment. Given such behaviors, we speculate that the abfrontal cells act as contact chemoreceptors. Yet spionids also use their palps to "monitor" the water column (Dauer *et al.*, 1981; Ferner and Jumars, 1999), and the lateral and

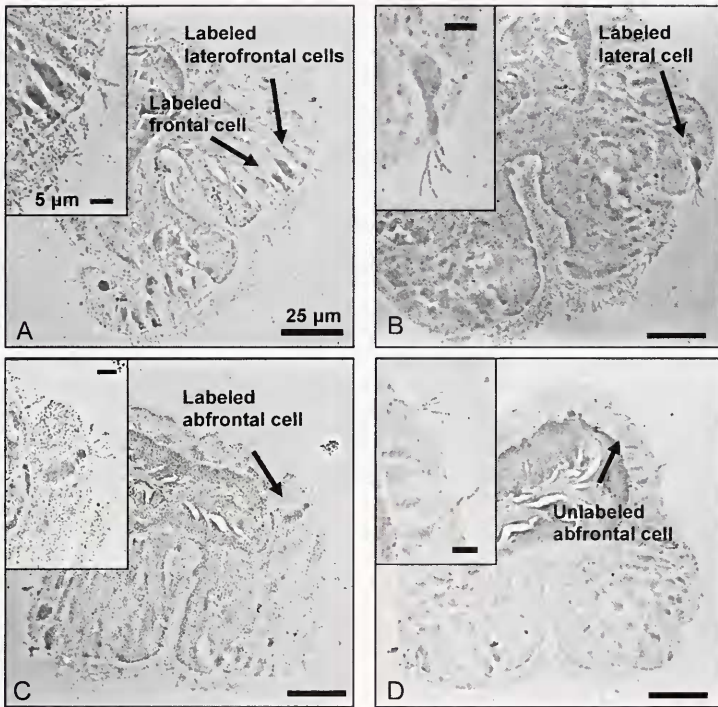


Figure 7. Labeling of *Dipolydora quadrilobata* palp cells by agmatine in the presence and absence of the amino acid mixture. (A) Semi-thin section from palp exposed to agmatine + amino acids showing labeled laterofrontal and frontal cells; scale = 25 μm . Inset shows the same cells at higher magnification; scale = 5 μm . (B) Semi-thin section from palp exposed to agmatine + amino acids showing labeled lateral cell; scale = 25 μm . Inset shows the same cell at higher magnification; scale = 5 μm . (C) Semi-thin section from palp exposed to agmatine + amino acids showing labeled abfrontal cell; scale = 25 μm . Inset shows the same cell at higher magnification; scale = 5 μm . (D) Semi-thin section from palp exposed to agmatine without amino acids (control), showing unlabeled abfrontal cell and its cilia projecting from the cuticle; scale = 25 μm . Inset shows the same cell at higher magnification; scale = 5 μm .

abfrontal sensory cells might also function as distance chemoreceptors.

Among invertebrates, preliminary studies suggest that gustatory ("taste," or contact chemosensitive) and olfactory ("smell," or distance chemosensitive) sensory neurons show a considerable amount of structural similarity (Dionne and Dubin, 1994). For example, two populations of sensory cells are distributed on the sensory tentacles of the nudibranch *Phestilla sibogae*. The intrapithelial sensory cells (contact or short-distance chemoreceptors) and subepithelial sensory cells (olfaction) have distinct spatial distributions, axonal organizations, and sensitivity to chemical cues, yet have very similar electrophysiological and pharmacological characteristics (Boudko *et al.*, 1997, 1999). Our experiments with *D. quadrilobata* demonstrate that the lateral and abfrontal sensory cells of the palps recognize both dissolved

(*i.e.*, lacking contact, this paper) and adsorbed (*i.e.*, contact necessary, Riordan and Lindsay, 2002) cues. The initial ultrastructural information reveals no significant differences among the groups of palp sensory cells (laterofrontal, lateral, and abfrontal). Characterization of the innervation patterns and immunoreactivity to serotonin and FMRFamide is ongoing.

Conclusion

Previous research established that selection of particles of certain sizes by spionid palps can be influenced by mucus adhesion strength and particle "stickiness" (Jumars *et al.*, 1982; Taghon, 1982; Dauer, 1985). Qian and Chia (1997) have speculated about a possible sensory role the palps may play in selective feeding, and putative sensory structures

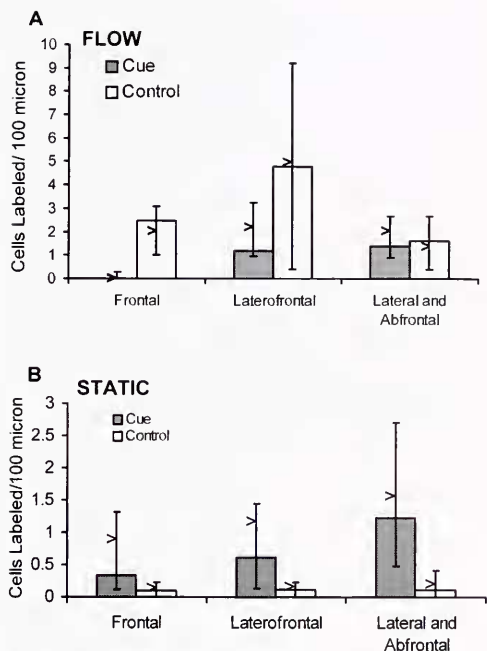


Figure 8. Number of cells per 100 μm of palp of *Dipolydora quadrilobata* that were labeled by agmatine in the presence (cue) and absence (control) of the amino acid mixture. (A) Results from the flow experiment using 20 mM agmatine. Numerous sections were collected from each of four control individuals and five treatment (cue) individuals; medians are presented, and error bars indicate the 75th and 25th percentile values. ">" indicates the value of the mean. (B) Results from the static experiment using 40 mM agmatine. Numerous sections were collected from each of six control individuals and six treatment (cue) individuals; medians are presented and error bars indicate the 75th and 25th percentile values. ">" indicates the value of the mean.

have been identified on the palps (Dauer, 1984, 1987, 1991, 1997; Worsae, 2001), but direct evidence linking sensory cells to selective behavior was lacking. This study presents the first physiological evidence that the peripheral sensory receptors on spionid polychaete palps detect chemical stimuli that elicit a selective feeding response. The percentage of peripheral cells that were labeled in the presence of amino acids varied by cell type (3% to 14%), and was greatest for the cells located on the lateral and abfrontal surfaces of the palps. This rate of labeling is similar to that observed for olfactory neurons in spiny lobster aesthetascs (0.5% to 4.6%, Steullet *et al.*, 2000). For spionid polychaetes, ultrastructural, positional, and now physiological evidence strongly suggests that the feeding palps are equipped with lateral and abfrontal sensory cells that function as chemoreceptors. Electrophysiological experiments will be re-

quired to determine whether these cells and the laterofrontal cells might also function as mechanoreceptors.

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